Altered Antigen Uptake and Distribution Due to Exposure to Extreme Environmental Temperatures or Sleep Deprivation[1]

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Mice were injected intraperitoneally with ^{35}S-labeled sheep red blood cell stroma and subjected to cold or hot environmental temperature extremes or sleep deprivation. While none of the hot temperatures had any consistent effect on the uptake and distribution of the antigen, cold exposure of one to three days duration resulted in a transient increase in uptake by peritoneal cells and a persistent decrease observed in spleen and liver. Three day sleep deprivation resulted in decreased uptake by peritoneal cells and spleen with a concomitant increase in liver. The results are discussed in relation to periodicity and seasonal variation of phagocytic activity as well as disease defense mechanisms.

INTRODUCTION

Environmental stressors such as temperature extremes and high altitude have been shown to have a definite effect on resistance to infectious disease (10) and the immune response (9, 14). With respect to certain particulate immunogens, uptake and subsequent processing of the antigen are integral steps in the immune response (5, 6). In this regard, nonspecific phagocytosis of etiologic agents has long been considered an important component of an animal's disease defense mechanisms.

The purpose of this investigation was to determine if the environmental stressors of temperature extremes had any influence on the uptake and distribution of parenterally administered particulate antigens. In addition, the effects of sleep deprivation and its associated fatigue on the same parameters was determined. Knowledge of such changes could be valuable in understanding alterations of the immune response observed in cold-exposed animals (9).

[1]In conducting the research described, the investigators adhered to the *Guide for Laboratory Animal Facilities and Care* as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care, of the Institute of Laboratory Animal Resources, National Academy of Science—National Research Council.

The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the view of the Department of the Army or the Department of Defense.

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MATERIALS AND METHODS

Animals and Housing. Random bred mice were obtained from the Charles River Breeding Laboratory, Inc., North Wilmington, Mass. Female mice, 8 to 14 weeks of age, were used in groups of 6, housed in stainless steel cages with wire mesh floors, and had free access to food and water.

Environmental Conditions. Temperature extremes were controlled to within ± 0.5 C in Lab-Line walk-in environmental rooms. Mice were sleep deprived by continual confinement on pedestals (8) 2.2 cm in diameter surrounded by water 4.5 cm in depth. Lighting in all the rooms was on a 12 hr on, 12 hr off cycle. Water bottles and food were accessible to the animals through overhead screening.

Preparation of Stroma. ^{35}S-labeled sulfanilic acid was prepared from $H_2{}^{35}SO_4$ (New England Nuclear Corp., Boston, Mass.) according to the method outlined by Campbell *et al.* (1). The preparation of stroma and the diazotization of the ^{35}S-sulfanilic acid was carried out according to the procedure of Ingraham (7).

Samples for Scintillation Counting. Animal tissues weighing 0.05 to 0.3 gm were digested in 0.3 ml perchloric acid in 0.6 ml hydrogen peroxide for one hour at 80 C in a sand bath. The digestion tubes (18 x 150 mm) were covered with marbles to obtain proper refluxing during digestion without loss by evaporation. Digested samples were cooled and quantitatively transferred to plastic counting vials by rinsing the tubes with 8 ml Aquasol (New England Nuclear). To each vial was added 4.0 ml of 0.4 M acetic acid to gel the sample and to suppress any chemiluminescence from the H_2O_2. The radioactivity was determined in a Beckman LS-233 scintillation counter using the ^{14}C + ^{3}H isoset. Counts per minute (CPM) corrected for quench are hereafter referred to as decays per minute (DPM).

Fluorescent Antibody (FA) Preparation and Tissue Staining. Whole sera from rabbits hyperimmune to sheep red blood cells, were thrice fractionated at 1/3 saturation with ammonium sulfate and dialyzed against 0.5 M sodium carbonate buffer, pH 9.0. For the conjugation reaction, 500 mg of γ-globulin in 15 ml were combined with 27.5 ml saline and 7.5 ml carbonate buffer at 4 C. Twenty-five mg of fluorescein isothiocyanate (Calbiochem, Los Angeles, California) were added slowly with constant stirring; mixing was continued for 18 hr in the cold. The material was then exhaustively dialyzed against 0.01 M phosphate buffered saline (PBS), pH 7.0. Nonspecific fluorescence was removed by absorption of the FA with 100 mg mouse liver tissue powder/ml FA. Frozen tissue sections were rinsed in cold PBS for 10 min and stained with FA for 20 min. Specimens were examined and photographed on a Zeiss Ultraphot II Microscope using BG38/3 excitation filters and barrier filter 50.

Method of Harvesting Peritoneal Cells. After the mice were sacrificed by cervical dislocation, the skin over the abdomen was reflected back without damaging the peritoneal membrane. Four ml of saline containing 1% calf serum were injected into the peritoneal cavity. The abdomen was massaged for 30 sec and at least 2 ml of fluid were withdrawn using an 18 gauge needle. Peritoneal wash fluid was centrifuged (1000 x g for 5 min) to sediment cells but not stroma. Before and after

centrifugation of the fluid, an aliquot was removed and counted. In isotope-injected mice, the difference between the counts of the cell suspension and the supernatant fluid was assumed to be the activity taken up by the peritoneal cells.

Statistical Analysis. Populations of animals were compared by analysis of Student's t distribution as outlined by Spiegel (12).

RESULTS

Determination of Organs Demonstrating Specific Localization of Stroma and Determination of Appropriate Sampling Times. Mice were injected i.p. with 0.4 ml of either 10% (v/v) ^{35}S-labeled stroma or ^{35}S-sulfanilic acid in order to determine which organs showed a specific uptake of stromal antigen and which organs had radioactivity due only to the passive accumulation of the injected material. Initially, spleen, liver, lung, kidney, intestine, blood, urine, and peritoneal cells were examined. Animals were sacrificed and samples removed at intervals from one to 72 hr following injection. A significant difference between stroma and sulfanilic acid uptake by a particular organ was taken to indicate specific localization in stroma rather than passive uptake resulting from blood flow. A significant difference in uptake between the stroma and sulfanilic acid was observed only in spleen, liver, and peritoneal cells. While there was a slight but consistent difference in the lung samples, the difference was not significant and reflected, to some extent, the activity found in blood.

Four hours after injection was found to be the time of maximum localization in stroma, spleen and liver. Localization of the labeled stroma in peritoneal cells was always optimal one hour after injection which also was the earliest sampling time. In all subsequent experiments, samples were taken at 1, 4, and 8 hours after injection.

Variability in the absolute DPM values was consistently encountered despite all efforts to minimize the degree of change from one experiment to another. Therefore, a control population of mice of the same age and weight was used in parallel with each of the studies reported.

Effects of Periodicity. Groups of 10 mice were injected with stroma at 4-hr intervals to cover a 24 hr period. In all instances, the animals were sacrificed 4 hr after injection and their peritoneal cells, spleen and liver were assayed for antigen uptake. The results are presented in Figure 1. Whereas there appears to be insignificant variation in the antigen uptake by peritoneal cells, uptake by spleen and liver is minimal during the nocturnal period. Considering this variability, 8 A.M. was chosen as the time to be used for the i.p. injections in all subsequent experiments.

Effects of Cold-Exposure. Experiments were designed whereby animals were injected with labeled stroma and immediately exposed to 8 C. A significant difference was never observed between this group and the 22 C control animals. Subsequently, the animals were exposed to the cold environment either one or 3 days prior to injection.

Following one day of cold-exposure, significantly less stroma was found to be localized in liver and spleen samples one hr after injection when compared to

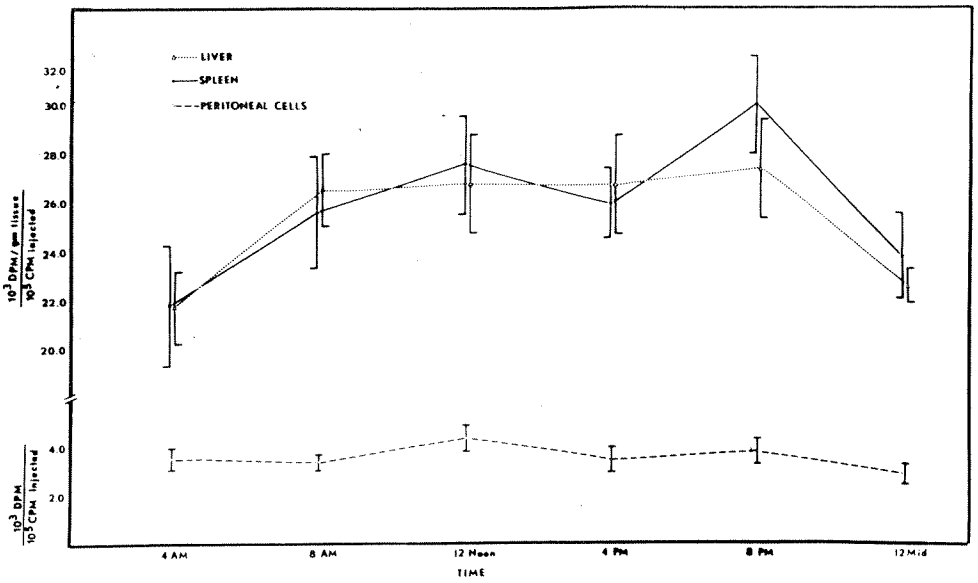

Fig. 1. Variation in uptake of ^{35}S-sheep cell stroma in mice as a function of time of injection. Each point is the mean of 10 animals and the bar represents ± S.E.

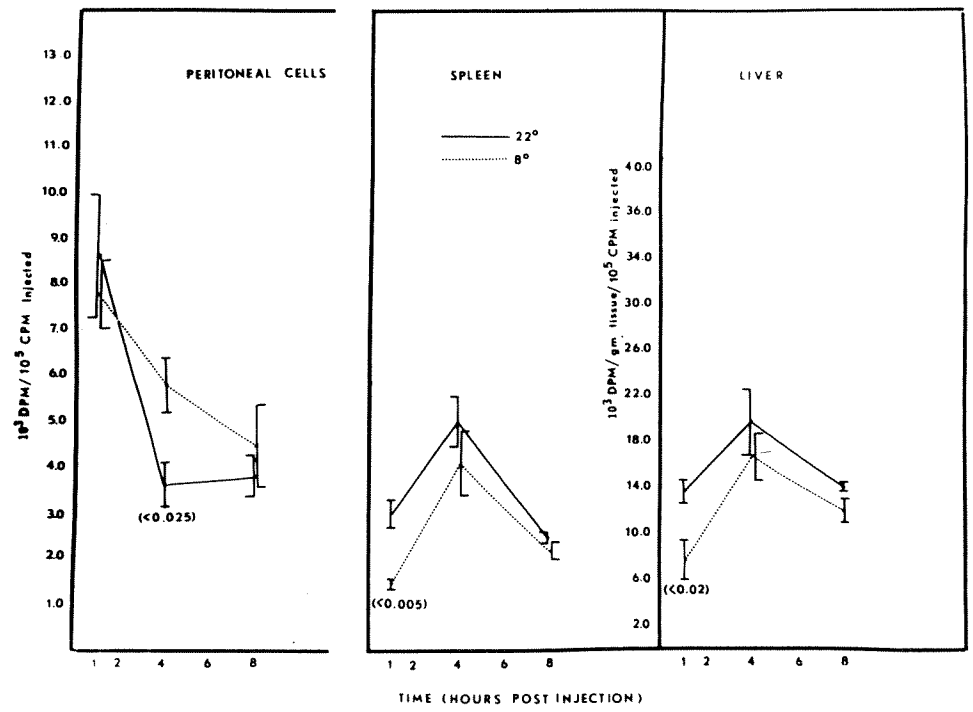

Fig. 2. Uptake of ^{35}S-sheep cell stroma in mice exposed to 8 C for one day prior to antigen administration. Each point is the mean of 6 animals and the bar represents ± S.E. 2P values determined from Student's t distribution are shown in parenthesis.

control animals (Figure 2). No differences of significance were observed in other tissue samples. However, there was significantly greater stroma present in the peritoneal cells 4 hr after injection as compared to control animals. The rate of stroma decrease in the peritoneal cells was less than in the control animals.

If the mice were exposed to the cold environment for 3 days prior to administration of stroma, the previously observed differences, with respect to peritoneal cells, were no longer apparent. Also contrary to the results obtained after one day of cold-exposure, 3 days of cold-exposure, prior to injection, resulted in significant decreases in stromal uptake in both spleen and liver at 4 hr, but not at one hr after injection as observed previously (Figure 3).

Effect of Heat-Exposure. If animals were given stroma and simultaneously exposed to the stress, there were no observed alterations in antigen uptake or localization as a result of an environment of 36 C and 80% relative humidity.

With respect to a one day exposure in the hot-wet environment prior to antigen administration, there was considerable variability in the uptake and localization of stroma in the spleen and liver. Significant differences could be observed in one of these organs in one particular trial and in the other organ or not at all in subsequent repeats of the same experiment. When differences were noted, spleen content was lower at one and 8 hr after injection and higher in liver at the same sample times.

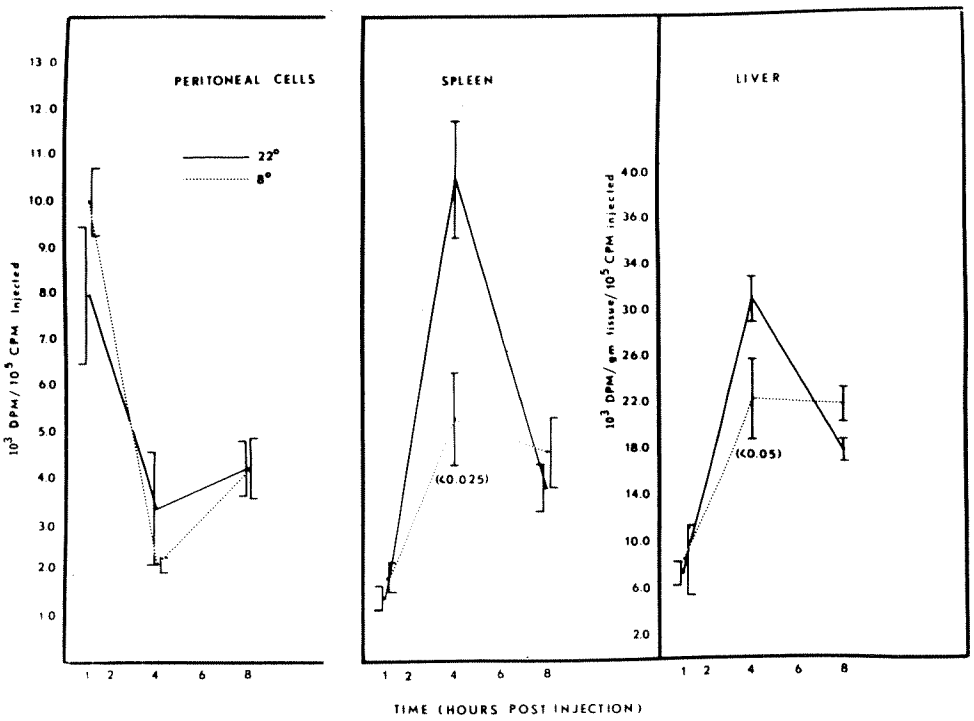

Fig. 3. Uptake of ^{35}S-sheep cell stroma in mice exposed to 8 C for 3 days prior to antigen administration. Each point is the mean of 6 animals and the bar represents ± S.E. 2P values determined from Student's t distribution are shown in parenthesis.

When mice were exposed to the hot-wet environment for 3 days prior to antigen administration, no significant changes in the uptake and localization of stroma were observed.

Effect of Sleep Deprivation. Only one elapsed time period was utilized in the sleep deprivation studies. The mice were placed on the pedestals in the morning and 3 days later were injected with stroma also in the morning. If the animals were allowed to remain on the pedestals for another 24 hr, the mortality rate began to rise drastically, mostly due to drowning. Samples were taken from the sacrificed animals in the same time sequence as in the previous experiments.

Significantly less stroma was localized in peritoneal cells after 4 hr when compared to control animals (Figure 4). Less activity was found in spleen samples also taken at 4 hr. However, liver samples had significantly more localization of stroma after both 4 and 8 hr. No significant changes were observed in all the other samples examined.

Control Studies. White blood counts and differential counts as well as peritoneal cell counts were done on noninjected cold-exposed, heat-exposed and sleep-deprived mice for comparisons with control animals. Only one significant difference was found in all the parameters measured. Mice housed at 36 C for 3 days prior to the

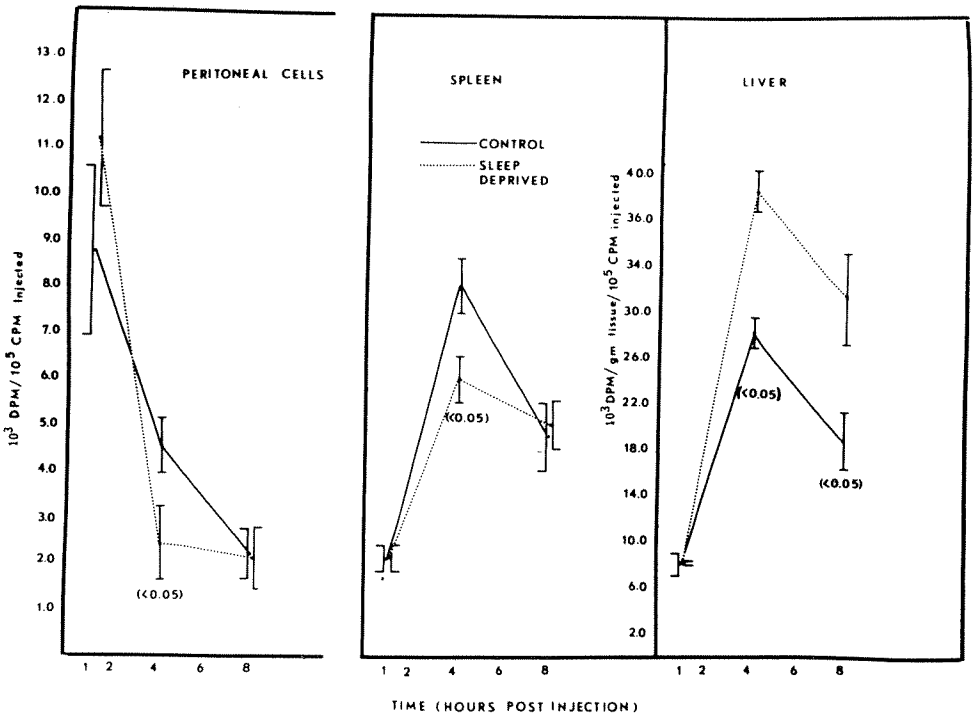

Fig. 4. Uptake of ^{35}S-sheep cell stroma in mice sleep-deprived for 3 days prior to antigen administration. Each point is the mean of 6 animals and the bar represents ± S.E. 2P values determined from Student's t distribution are shown in parenthesis.

injection of stroma had significantly fewer peritoneal cells than did the control population at 22 C or cold-exposed animals.

Rectal temperatures were recorded on cold- and heat-exposed animals. The mice were neither hypothermic nor hyperthermic as a result of the altered environments.

Fluorescent Antibody (FA) Studies. There was some concern that the ^{35}S-label would come off the stroma *in vivo* and, therefore, the quantity in terms of DPM associated with the organ samples would not be indicative of stromal uptake and localization. Fluorescent antibody studies were done on frozen sections taken at each time interval from the control animals. A correlation between FA binding and DPM was made by comparing a subjective observation of fluoresence intensity and CPM. The study indicated that fluorescence intensity, as judged independently by two observers, correlated in every case with DPM.

Fluorescent antibody-stained sections of spleen and liver, as well as stained smears of peritoneal cells, were examined for localization of stroma. Stroma was found to be localized predominantly in the trabeculae and white pulp of the spleen. In liver sections, fluorescent staining was confined to the lining of the sinusoids. Peritoneal cells containing stroma stained uniformly and almost all of the cells appeared to contain stroma.

DISCUSSION

We have demonstrated that ^{35}S-labeled stromal antigen uptake and distribution may be altered as a result of sleep deprivation and exposure of mice to cold temperatures. Exposure to a combination of heat and high humidity did not result in overt changes in antigen uptake and distribution; however, after a 3 day exposure, it did cause a significant decrease in the population of peritoneal cells.

Unlike previous studies on antigen uptake, the antigen was administered i.p. rather than i.v. Therefore, we would expect that any direct effects of the stressors used would be reflected in alterations in peritoneal cell uptake. Alterations in spleen and liver uptake should be considered indirect indicators of stress effects since distribution to, and uptake by, these organs is most probably dependent upon blood flow from the mesenteric vessels.

In the 2 cold-exposure experiments, a definite shift in the cell and organ response to stress was noted. Following one day of cold-exposure, the decrease in stromal uptake by spleen and liver was apparently partially compensated by an increase in uptake by peritoneal cells. However, the compensation failed to account for the quantitative difference between cold-exposed and control animals. With the exception of preliminary experiments in which we examined spleen, liver, lung, kidney, intestine, blood, urine, and peritoneal cells, no attempt was made to account for the total radioactivity contained in the original i.p. injections. Following 3 days of cold-exposure, whatever factors are affected by one day cold-exposure have been modified by the end of this time.

Perhaps there have been alterations in blood flow resulting from cold-exposure. Alternatively, there could have been some direct damage to the phagocytic cells of these organs as a result of humoral or cortical steroid changes induced by cold-exposure. We have no evidence to support or reject this possibility. It is unlikely that the phagocytic cells of cold-exposed animals are more efficient in breaking

down antigen or we would have observed decreased localization of stroma in all samples, not just at one time interval. It may reflect, however, a more rapid overall catabolism by the cold-exposed animal.

Although we had checked the periodicity of uptake on control animals, we did not do this on any of the stressed animals; therefore, the shift of decreased spleen and liver localizations from one hr to 4 hr post-injection could be an alteration in the periodicity of uptake brought about by prolonging the cold-exposure. Periodicity of phagocytic activity in the control groups exhibited seasonal variations as previously observed in mice (11) and rabbits (4). It could be that what we demonstrated in spleen and liver with respect to cold exposure is in part an extension of the normal seasonal variation.

With respect to heat-exposed mice, one can draw either of 3 conclusions. The peritoneal cells from the 3 day heat-exposed animals were more efficient in their ability to take up antigen, or they retained the antigen longer without breaking it down, or the quantity of the peritoneal cell population was not a limiting factor and thus there was truly no difference between control and heat-exposed animals. We tend to favor the last alternative, especially considering the lack of significant difference in either the spleen or the liver.

The results of studies on sleep-deprived animals demonstrated a clear difference in the manner in which the stroma was taken up and distributed. It appears that there was a direct effect upon the peritoneal cells in that they had less antigen 4 hr after injection. Similarly, the spleen had less stroma at the same time interval. However, the liver had higher quantities of antigen both 4 and 8 hr after injection. Therefore, blood flow may be a factor in the alterations seen in sleep deprivation considering the difference between the spleen and liver which suggests there could be a shunt of blood flow from the spleen to the liver.

The information obtained in these studies may aid in understanding the ultimate effect of these stressors on the resulting immune response as well as to help evaluate the role of nonspecific phagocytosis in the clearance of infectious agents. For instance, other authors have demonstrated that cold-exposed animals have an earlier onset of antibody production and a higher titer (9), a more rapid fall in titer (14), and ultimately an apparent adaptation to cold (3). It is conceivable that the increased uptake of the locally injected stroma by peritoneal cells, observed following one day cold exposure, could result in an increased antibody titer. However, this must remain as speculation since we did not measure antibody titers in any of our animals. It is also difficult to relate our results to those of other authors in that both the animal species and route of antigen administration differ.

Stark (3) reported that animals exposed to a warm temperature (30 C) have reduced antibody titers. Again it is difficult to relate our observations to that of Stark in that the route of antigen administration differed although the animal species were the same. If, after intravenous administration of stroma, no differences were observed in antigen uptake, then one could speculate that the decreased antibody titers observed by Stark would be due to some event beyond antigen processing. One such event would be antibody catabolism. Preliminary results in our laboratory (2) indicate that the decay rate of antibody in mice exposed to a hot-wet environment is decreased.

In light of the results of these studies, it would be of interest to know if there are any alterations in the ability of phagocytic cells to destroy bacteria.

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